

First report of *Erysiphe bunkiniana* from Pakistan

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ABSTRACT—During August–September 2018, powdery mildew symptoms were observed on both surfaces of leaves of *Isodon rugosus* in the Abbottabad and Malakand districts of Pakistan. The causal agent was identified as *Erysiphe bunkiniana*, based on its asexual morph and chasmothecia, and its identity was confirmed by molecular data. This is the first Pakistani report of *Erysiphe bunkiniana* and the first sequence obtained for this powdery mildew species.

KEY WORDS—*Erysiphaceae*, *Lamiaceae*, new record, ITS sequence

Introduction

Powdery mildews are biotrophic, often highly specialized pathogens, attacking nearly 10,000 vascular plant species (Braun & Cook 2012). More than one powdery mildew species can parasitize a single host plant species. The 872 species described by Braun & Cook (2012) form a characteristic white, talcum powder-like coating on the surfaces of different host plants, including economically important crops and ornamentals. Accurate identification is essential for effective control; however, these obligate biotrophs pose problems for taxonomists, as many species lack clear morphological characters to distinguish them, are difficult to culture, and therefore require molecular techniques to discriminate between them. The increasing application of molecular methods to the study of powdery

mildews has led to an increasing number of described species (Ellingham & al. 2019). From Pakistan, only 10 genera and 42 species of *Erysiphaceae* are known (Ahmad & al. 1997, Anwar & al. 2020, Riaz & al. 2020, Afshan & al. 2021). Currently, research using morphological and molecular methods is underway to explore this group of fungi. Many new records (as well as new species) are expected in the future.

During 2018, a powdery mildew fungus was collected on leaves of wrinkled-leaf isodon (*Isodon rugosus*) in the districts Abbottabad and Malakand, Pakistan. The infected leaf surfaces were covered with white powdery mycelial masses, conidiophores, conidia, and chasmothecia. After careful morphological and molecular analyses, the fungus was identified as belonging to the genus *Erysiphe*.

Materials & methods

Sample collection

During phytopathological surveys in August–September 2018, we observed powdery mildew infections on leaves of *Isodon rugosus* in the district Abbottabad and Malakand, Khyber Pakhtunkhwa, Pakistan. The infected plants were shade dried on blotting paper and protected in brown envelopes for future research. The specimens were deposited at the herbarium of the University of Peshawar, Khyber Pakhtunkhwa, Pakistan (PUP) and Institute of Botany, University of the Punjab, Lahore, Punjab, Pakistan (LAH).

Morphology

The infected leaves were examined under a Labomed CSM2 stereomicroscope, and slides were prepared in lactic acid. We examined the hyphae on the host, hyphal appressoria, conidia, conidiophores, and chasmothecia (including asci and ascospores) under a Nikon YS 100 microscope. Twenty measurements per structure were recorded using a Lomo Filar Eyepiece Micrometer AM9-2-15x on a Zeiss microscope. Micrographs were made using a HDCE-5X digital camera.

DNA extraction, PCR amplification, phylogenetic analysis

Powdery mildew infections were scraped off fresh fungal specimens with sterile razor blades, ground in liquid nitrogen, and stored in Eppendorf tubes at 18 °C. DNA was extracted using GeneJET Plant Genomic DNA Purification Mini Kit #K0791 according to the manufacturer's instructions. The Internal Transcribed Spacer (ITS) region was amplified using PMITS1/PMITS2 primers (Cunnington & al. 2003) and then commercially sequenced by Tsingke in China. Raw sequence data were edited using BioEdit (Hall 1999). The ITS sequences were BLASTn searched against the GenBank database (www.ncbi.nlm.nih.gov) and aligned using Muscle E multiple alignment tool within MEGA X (Kumar & al. 2018). Forty-two ITS sequences were analyzed using the maximum likelihood (ML) method based on the GTRCAT model

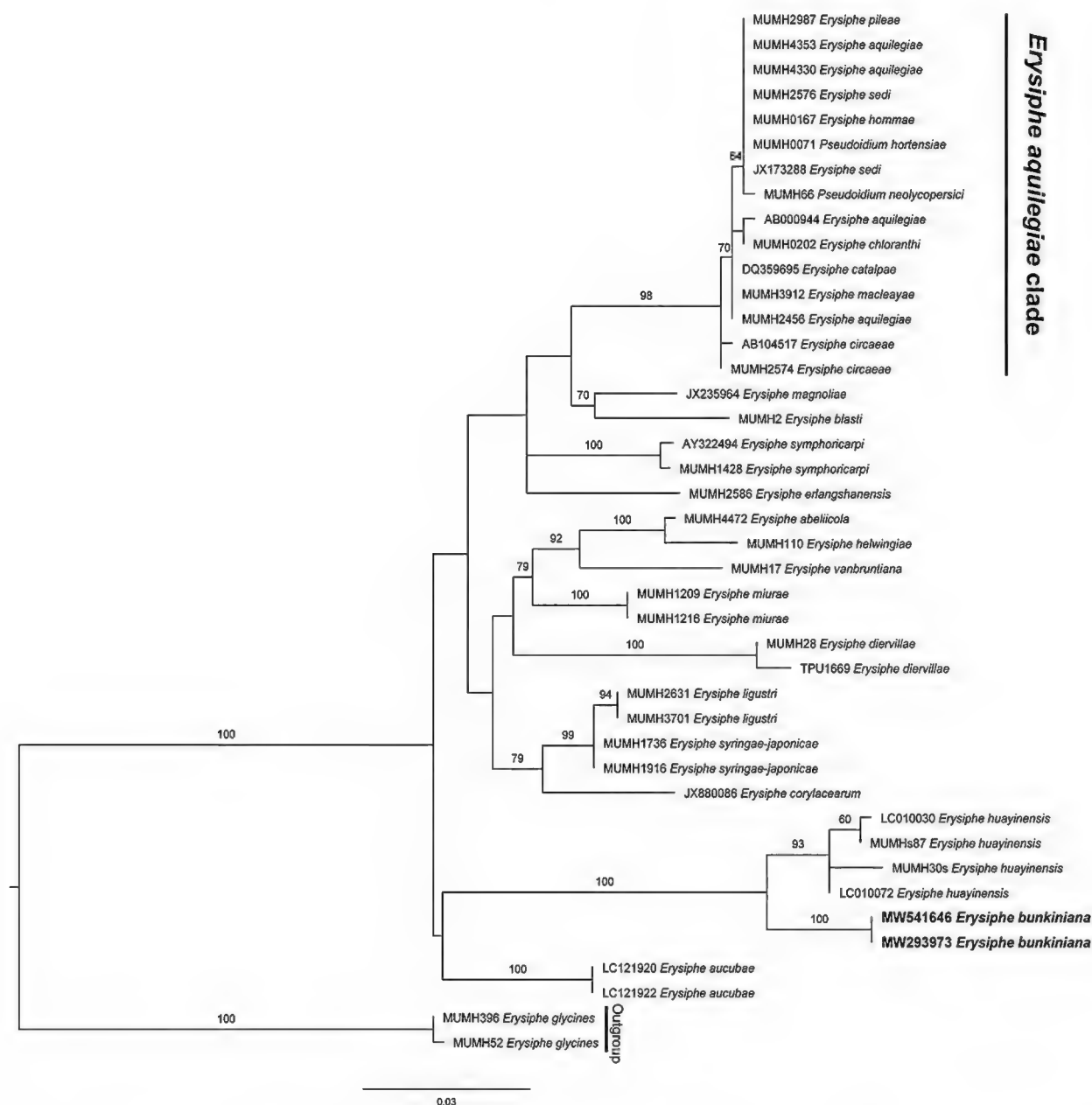


FIG. 1. Phylogenetic analysis of the ITS region for 42 *Erysiphe* sequences, with *Erysiphe glycine* as outgroup. The evolutionary history was inferred by using the Maximum Likelihood method based on the GTRCAT model in RAxML-HPC2. Bootstrap support values >60% are cited on nodes. Amplified sequences of *Erysiphe bunkiniana* from Pakistan are in bold.

in RAxML-HPC2. The final phylogram was visualized in FigTree.v1 4.4. and edited using Adobe Illustrator. After elimination of gaps and missing data, the final dataset comprised 614 positions. *Erysiphe glycine* served as the outgroup. All sequences were aligned and trimmed at conserved sites from both 5' and 3' ends.

Phylogenetic results

The final ITS dataset comprised 614 positions, and the phylogenetic tree is shown in FIG. 1; the percentage of trees in which the associated taxa clustered together is shown next to the branches, and the tree is drawn to

scale, with branch lengths measured in the number of substitutions per site. *Erysiphe bunkiniana* (FIG. 1) clusters with *E. huayinensis* (LC010080, LC010072, AB015914, LC010030), with a 95-bootstrap value.

Taxonomy

Erysiphe bunkiniana U. Braun, Feddes Repert. 91(7–8): 441 (1980) FIG. 2

MYCELIUM amphigenous, mostly epiphyllous, thick, persistent to evanescent, effuse and in white patches; HYPHAE hyaline, smooth, septate, thin-walled, about 2 µm diam. HYPHAL APPRESSORIA nipple-shaped to moderately lobed, 4–8 µm diam. CONIDIOPHORES arising from upper surface of mother cell, 65–103 × 9–10 µm; FOOT CELLS cylindrical, straight or (sometimes) curved, 13–36 × 6–11 µm, constricted at basal septum, followed by 1–3 shorter cells, forming conidia singly. CONIDIA ellipsoid-ovoid, doliiform or cylindrical, 24–35 × 11–17 µm, without fibrosin bodies; GERM TUBES terminal to lateral, short to moderately long, conidial appressoria somewhat swollen, alobate to lobate, tubes 3–7 µm wide. CHASMOTHECIA scattered to gregarious, globose to subglobose, light to dark brown, 90–129 µm diam.; PERIDIUM CELLS polygonal, 6–22 µm diam. APPENDAGES numerous, about 6–25 in number, arising in lower half of ascomata, mycelioid, simple, straight or frequently irregularly to subdichotomously branched, 14–173 × 6–11 µm, apex often pointed, rarely obtuse, 0.6–3 times as long as chasmothecial diam., 4–12 µm wide, septate, walls thin, smooth or fairly rough, hyaline or slightly pigmented below. ASCI 4–9 per chasmothecium, ellipsoid, obovoid, 42–70 × 30–50 µm, sessile or stalked, 4–8-spored. ASCOSPORES ellipsoid to ovoid, 16–24 × 11–18 µm, colorless or yellowish.

SPECIMENS EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA, Malakand Division, Malakand district, 844 m alt., on *Isodon rugosus* (Wall. ex Benth.) Codd (*Lamiaceae*), 19 September 2018, A. Anwar A#13 (PUP Bot.05; GenBank MW541646); Hazara Division, Abbottabad district, Mukshpuri, at 9200 m alt., on *I. rugosus*, 13 August 2018, N.S. Afshan & J. Majeed JP#04 (LAH36140; GenBank MW29397).

Discussion

In the NCBI BLASTn analysis our ITS sequences (MW541646 and MW293973) showed 97% identity with *Erysiphe huayinensis* R.Y. Zheng & G.Q. Chen (LC010080.1), having 99% query coverage and 0.0 E. value. Sequences of different *Erysiphe* taxa were obtained from GenBank to determine the phylogenetic position of the sample. A few gaps were deleted from the final file to get the optimal alignment for phylogenetic analysis. The aligned data set included 614 sites of which 455 were conserved, 155 were

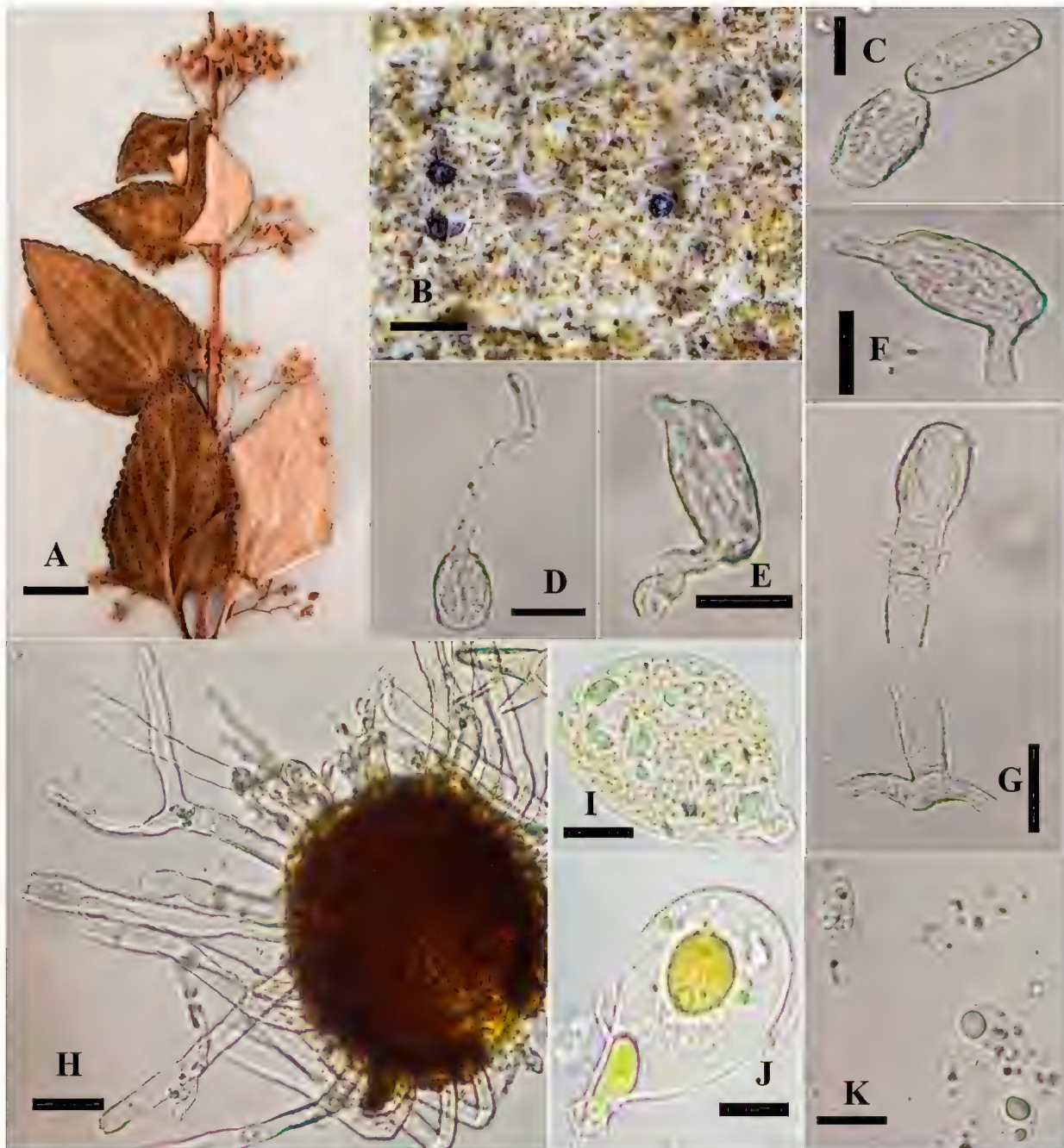


FIG. 2: *Erysiphe bunkiniana* (PUP Bot.05): A. Infected host plant, *Isodon rugosus*; B. Fungal mycelium under stereomicroscope; C. Conidia; D–F. Germinating conidia; G. Conidiophore; H. Chasmothecium; I, J. Asci with ascospores; K. Ascospores. Scale bars: A = 20 mm, B = 2 mm, C–F, I, J = 15 μ m, G, K = 20 μ m, H = 30 μ m.

variable, and 133 were parsimony informative. All ambiguously aligned gaps in the final aligned dataset were treated as ‘missing’.

In the phylogram, the new sequences retrieved from *Erysiphe* on *Isodon rugosus* (FIG. 1) cluster with *E. huayinensis* sequences (LC010080, LC010072, AB015914, LC010030) with a 93-bootstrap value. Morphologically, our

species agrees well with *Erysiphe bunkiniana* (Braun & Cook 2012), but we could not find any sequence data of this species in GenBank. Based on the morphological compliance of the Pakistani collections on *Isodon rugosus* with *E. bunkiniana* and the obvious sequence difference from *E. huayinensis*, which may also occur on *Isodon* spp., the identification of our powdery mildew specimens as *Erysiphe bunkiniana* is reasonable. Furthermore, *E. huayinensis* is well differentiated from *E. bunkiniana* morphologically by smaller ascomata and appendages that become thick-walled in the lower half. The apices of the appendages are often enlarged to subclavate (Braun & Cook 2012).

Erysiphe bunkiniana has previously been reported on *Isodon* spp. (*Lamiaceae*) from Asia (China, India, Russian Far East) (Fungus-Host Database <https://nt.ars-grin.gov>, 29 March 2021; Braun & Cook 2012). According to Liu & Braun (2009), it is difficult to distinguish *E. bunkiniana* from *E. rabdosiae* R.Y. Zheng & G.Q. Chen as these taxa were previously separated only based on the length of the chasmothecial appendages and the number of ascospores. According to Braun & Cook (2012), *E. rabdosiae* represented collections of *E. bunkiniana* with less developed appendages. *Erysiphe bunkiniana* is a new record for Pakistan, and this is the first Pakistani report of any powdery mildew on *Isodon rugosus*. Finally, the *E. bunkiniana* sequences obtained during the current research represent the first for this species.

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